A10. Keywords

Max. 5 keywords to describe the project activity.

Intestinal ecology, organic feed, probiotics, rainbow trout, sustainability

A11. Short project description/summary on objectives, activities, and expected results, both in Danish and English language (max 1500 characters, incl. spaces for both languages)

Formålet med OPTIFISH er at sikre optimale betingelser og høj overlevelse for regnbueørreder i økologisk akvakultur. Regnbueørreden er den dominerende opdrætsfisk i dansk akvakultur, hvoraf en mindre del produceres økologisk. Pt. er der ingen produktion af økologisk yngel, da en økologisk fisk igennem livsforløbet højst må behandles med antibiotika to gange, hvilket kan være svært at overholde pga. gentagne sygdomsudbrud specielt forårsaget af bakterien Flavobacterium psychrophilum på yngelstadiet. Endvidere vides det fra lakseopdræt, at vegetabilske proteinkilder (f.eks. sojamel) i foderet påvirker tarmslimhinden, hvor der ses betændelse med en efterfølgende svækkelse af immunstatus. OPTIFISH vil se på, hvordan økologiske fodertyper med indhold af forskellige mængder af marine og vegetabilske foderemner samt med og uden probiotika (mælkesyrebakterier) påvirker regnbueørredens tarm, den bakterielle tarmflora samt overlevelsen hos fisk i forbindelse med infektioner. Brugen af probiotika til ynglen forventes at resultere i øget sundhed. Resultaterne vil skabe en mere bæredygtig produktion ved en bedre udnyttelse af de tilgængelige økologiske naturressourcer og ikke mindst muligheden for, at erhvervet ved brug af den mest optimale fodring opnår en robust og sund fisk. Dette vil ikke bare kunne bruges ved opdræt af økologisk fisk, men også i det traditionelle opdræt. En mere robust og sygdomsfri yngel vil være nødvendig for en højere produktion, især i forbindelse med økologisk opdræt.

The aim of OPTIFISH is to optimize growth and survival for organic cultured rainbow trout, the dominant fish species produced in Denmark. A minor part of the rainbow trout is produced as organic fish. Currently there is no production of organic fry, as the classification organic only can be given to fish that have been treated with antibiotics no more than twice in a lifetime. This is hard to achieve as recurrent disease outbreaks, especially with the bacterium Flavobacterium psychrophilum, are seen during the fry stage. A further challenge known from salmon culture is that diets with high plant contents cause enteritis and injury to the intestine, which will affect the absorption of nutrients, affecting the overall health status and welfare of the fish. The result is a higher risk of disease following exposure to pathogenic microorganisms. OPTIFISH will investigate how organic diet types with varying amounts of fish and plant sources as well as with or without probiotics (lactic acid bacteria) will affect the intestine, the intestinal microbial flora and survival rates of rainbow trout following exposure to pathogens. The use of probiotics for fry are assumed to result in a higher health level. The overall result will be a sustainable production with an optimal utilization of the available organic ressources as well as the scope for the industry that they by using the optimal diet type will achieve a robust and healthy fish, something that can be achieved not only in organic but also in traditional farming. A robust and disease-free fry is the most important factor for a higher production in organic aquaculture in the future.

A12. Project description

(All parts of A12 must be filled out. Use "Garamond" as font, and font size 12, single spaced)

A12.1 The project objectives (2-3 lines). The aim of the project is to elucidate how probiotics as well as different feed types with varying amounts of marine versus plant protein and oil sources (of organic origin), affect the health status and welfare of farmed rainbow trout, thus contributing to ensuring optimal conditions and high survival rates for organic fish.

A12.2 The background and idea (hypotheses) incl. the national and international "state of art" and incl. references relevant for the section (max. ¾ page). Rainbow trout is the dominant fish species produced in Danish aquaculture with an annual production of 33,000 tonnes from approximately 250 freshwater farms. Freshwater aquaculture in Denmark is in a transitional phase. There is a continuing increase in the production of organic fish, but a stop is the strict regulation on treatment of disease outbreaks in organic fish. A more robust and healthy fry will be a solution addressed by OPTIFISH. Traditional fish farms are based on e.g. earth ponds, with water intake from streams. Here production expansions have not been possible due to very strict environmental requirements. In later years, a new farm type has been implemented based on a more sustainable production with water intake from bore-hole or drain, using a high level of water recirculation as well as extensive wastewater treatment, creating a unique opportunity for production expansion on a farm with minimal environmental impact. Also this expansion in production demands a robust and disease-free fish.

Feed for rainbow trout aquaculture has traditionally been based on marine resources such as fish meal and fish oil. Because of a shortage of marine resources as well as the growing production of farmed fish, the feed industry has been forced to partially exchange fish meal protein with proteins derived from plants, like soy bean meal. This has been shown to affect the salmonid intestinal mucosa, in some cases resulting in intestinal inflammation (Bæverfjord & Krogdahl 1996; Refstie et al. 1997; Burrells et al. 1999; Krogdahl et al. 2000; Bakke-McKellep et al. 2007; Iwashita et al. 2008). In addition, plant-based dietary proteins have been associated with changes in disease susceptibility in salmon and it has been suggested that these special diet types weakens the immune status of the fish. The investigations have primarily been done on larger fish.

One major cause for losses in Danish freshwater fish farms is the fry disease rainbow trout fry syndrome (RTFS). RTFS is caused by the bacterium Flavobacterium psychrophilum. It has been estimated that the mortality among fry due to RTFS averages about 34 percent (around 88 million dead fry), corresponding to 18 million DKK in direct losses (1998 figures; Jensen et al. 2003). Often recurrent disease outbreaks are seen giving rise to the need for recurrent antibiotic treatments. This stresses the importance of optimal health status in the fry, and experiences of the fish farmers suggest that the diet type is an important factor for disease development. Enteric redmouth disease caused by Yersinia ruckeri is also an economically important disease which causes problems in rainbow trout fry as well as larger fish.

The aim of the project is to reduce the occurrence of bacterial infections in Danish aquaculture by applying newly gained knowledge on the influence of organic diet types on the establishment of a normal microbiota in the intestine and the immune system. Further, how probiotics (commercially available in fish diets) influence the intestinal microbiota, also in connection with infections. The description of the intestinal microbial flora in fish has primarily been done by traditional bacteriology, but the introduction of molecular techniques will give a more precise as well as a more diverse picture of the bacterial composition of the intestine. It will also be investigated how an experimental infection of the fish will influence this microbiota and if different infection levels are seen among the diet types, as well as if probiotics as feed additives might reduce mortalities. The goal will be a robust fingerling treated less than twice with antibiotics, thereby not loosing its predicate as organic, by the help of the most optimal organic feed as well as probiotics as feed additives.

A12.3 The projects contribution to solving important challenges for the organic food, agriculture and aquaculture sectors and the general political goals regarding food, agribusiness and environment as expressed in the governments Green Growth programme. Including an explanation of the projects focus on respectively the entire product/value chain or selected parts here of (e.g. primary production, processing, trade and transport) – max. ½ page. Trout cannot be classified as organic, if they are treated with antibiotics more than twice. Currently organic fry is not produced, as there are high risks of disease outbreaks with the bacterium Flavobacterium psychrophilum during the fry stage resulting in several antibiotic treatments of the fry. OPTIFISH will focus on this important disease by studying how different organic diet types will affect a possible infection with this pathogen at the fry stage. The addition of probiotics is expected to be a strong prophylactic measure to improve health in fry as well as the possibility of keeping mortality levels during potential disease outbreaks as low as possible, as it is expected that the probiotics will be able to survive in the digestive tract and thereby inhibiting the colonization of potential pathogens. In conclusion the use of probiotics is expected to prevent or limit the need for antibiotic treatment in production of rainbow trout fry.

A12.4 The projects innovative value, relevance and effect including the specific barriers and development potential for the organic sector the project will solve and/or support (max. ½ page).
The project will focus on the early life stages of rainbow trout, stages where a healthy condition of the fish is fundamental for optimal production efficiency of the fish, especially in organic aquaculture. It is expected that the gained knowledge on the influence of diet types on the structure and function of the gastrointestinal tract of the fish will lead to an optimisation of feed quality. Furthermore, probiotics are expected to be of great health benefit to the fry e.g. inhibiting the colonization of potential pathogens. Both migh help to prevent diseases and thereby lower the amount of antibiotics used in Danish aquaculture, which amount to 1.4 tonnes in 2008.
The project will strengthen aquaculture research in Denmark and is very relevant for the organic fish industry as well as for national and international research. The project will result in essential knowledge with a high utility effect in trout production. The project will create increased possibilities for growth and optimal health in the rainbow trout production, and it will result in important knowledge relating to diet ingredients that should be given priority in the future.
Practising veterinarians within the field of fish farming have described a phenomenon among rainbow trout, where high mortalities are seen among fry just after first feeding. It seems that the bacterial flora in the intestine of these fry is dominated by only a few bacterial species that are not otherwise known to cause disease. The project will elucidate interactions between diet, probiotics, microbiota population composition and diversity and link this to disease susceptibility. It is expected that the fish health also will be improved if the diet is better adapted to the intestine and intestinal microbiota of the fry.
Thus the OPTIFISH project will contribute to knowledge of optimal diet composition for a healthy fish and enhance animal welfare. It will be equally important for a future evaluation of the fish as a healthy product for human consumption.

A12.5 Description of activities, methods and expected results divided into work packages with clear denotion of which activity the applicant consider to be either Research, Development or Demonstration. The coherence between work packages must be clearly described and the relation between activities and the tables with milestones and deliverables must be logical and consistent. Moreover, the primary target groups should be clearly identified with a description of how these will be met by the project (max. 1 page per WP and max. 3 pages in total).
The project will be divided into work packages (WPs), where the first WP covers the management of the project whereas the following WPs cover the experimental and laboratory work.

WP 1 – Management (partner 1)
The different WPs will be coordinated. E.g. samples from fish taken during feed experiment in WP 2 will be used for the investigations done in WPs 3 and 4. For more information on project management see section A12.9.

WP 2 – Feed experiments with fry (partner 4)
Broodstock of rainbow trout will be stripped and eggs fertilized according to normal procedures at the rearing facilities. Rainbow trout fry will be divided in groups, and fish in each group will be fed different diet types (commercially available as well as specifically formulated; including inclusion of probiotics) from first feeding. Samples will be taken continuously from the different groups and they will be used for WPs 3 and 4.

WP 3 – The bacterial microbiota in the intestine (partners 2 and 1)
The bacterial flora in the intestine in fish from the different diet groups will be compared by the use of traditional bacteriology as well as by molecular methods using 16S rRNA gene PCR combined with next generation sequencing. Thus gut microbiota, probiotic microorganisms and pathogens will be identified and quantified. The latter techniques will also be used in a metagenomic approach to assess the metabolic capability of the microbiota in fish fed the different diet types and fish from the experimental infection trial in WP 5. Sequence analysis will be done using the software CLC Genomic Workbench running in-house at DTU Vet.
The location in the intestine of the pathogenic bacteria (F. psychrophilum and Y. ruckeri) as well as probiotic bacteria will be studied by fluorescent in situ hybridisation in combination with confocal laser scanning microscopy and image analysis. Bacteria from the intestinal microbiota located close to intestinal lesions or the known disease-causing bacteria will be isolated by laser capture microdissection and identified by PCR and 16S rRNA gene sequencing.

WP 4 – Immunological investigation of the intestine (partners 3 and 1)
Samples from experimental fish from WP 2 will be used for the studies. The immune response will be studied by measuring leucocytes and the concentrations of total protein, albumin and globulin in plasma from blood samples. Tissue (head kidney, spleen, liver, intestine) will be analyzed for expression of relevant genes to elucidate the importance of both innate and adaptive parameters by quantitative RT-PCR. Furthermore samples will be screened for acute phase reactants. Expression of the complement factors and collectines like MBL as well as their receptors will be measured.

WP 5 – Investigations of fish health (partner 1)
A sub-set of fish from the different diet groups will be experimentally exposed to the bacterial fry pathogens F. psychrophilum (rainbow trout fry syndrome) and Y. ruckeri (enteric red mouth disease). During the infection experiments samples will be taken for use in WPs 3 and 4. The experimental infections will be done according to current legislation on experiments on animals.

The ethical aspects of the research.
It will be necessary to do experimental challenges in order to study the susceptibility to certain bacterial pathogens in groups fed the different diet types. Best husbandry practises and experimental treatments, including the use of anaesthetics, will be used in order to minimize suffering in these experiments. The project participants hold the necessary authorizations to carry out the experiments described in this project, according to Danish and European legislation. Similarly, the research facilities in which the experiments will be undertaken hold the appropriate authorizations. The experimental infection is necessary and of critical importance for the understanding of the pathogenesis and the potential preventive effect of different diet groups and probiotic treatment.

A12.6 Description of how it will be ensured that the project results can be implemented in practice and perhaps commercialized (max. ½ page).

The participition of a fish feed company in this project will ensure that project results will be commercialized if the results point in that direction. The probiotic is already commercially available from the company and it will be very important for both the company and the aquaculture industry to identify a diet that will prevent or reduce disease incidence in organic rainbow trout.

A12.7 Description of possibilities for a general utilisation of the results (max. ½ page).

The results can be used in both organic aquaculture as well as traditional rainbow trout farming. Furthermore, the project will result in increased knowledge regarding the relationship between diet and disease and a detailed knowledge of the influenze and importance of the composition of the microbiota and the host response.

A12.8 Description of the coherence between the research, development and demonstration activities in the project, including involvement of relevant users of the results (max. ½ page).

OPTIFISH is primarily a research project, where the aquaculture industry, represented by the feed company BioMar A/S and the Danish Aquaculture organization, are important and essential participants.

A12.9 Project organisation, management and administration (max. ½ page).

The project will be a collaboration among 5 partners that all are experts within their areas and together these partners possess a unique combined expertise, combining both research and knowledge of practical aquaculture (Partner 1: bacterial diseases and experimental infection in fish; Partner 2: interaction between feed, the intestinal microbiota, probiotic microorganisms and pathogens; Partner 3: fish immunology and pathogen/host interactions; Partner 4: fish feed production; Partner 5: knowledge of practical aquaculture). This multidisciplinary approach involving a broad range of experts ensures decisive results for a successful outcome of the project.

The project is divided into work packages (WPs), in which collaborators will have sole or shared responsibility with other collaborators (please see the description of the WPs A12.5). There will be a management team that will include all the involved participants. The project applicant senior scientist Lone Madsen will have the daily management of the project in collaboration with senior scientist Inger Dalsgaard.

The applicant has management experience from a Danish Research Council grant in 2001 and has been coordinating the National Reference Laboratory for mollusc diseases in Denmark since 2002. The applicant has experience with experimental infection models from several projects, including her PhD project, where the target fish pathogen was F. psychrophilum.

At biannual meetings with all project participants results and strategies will be presented and discussed and the further progress of the project will be organized.

A12.10. The technical competences of the partners and their contribution to the project including how they complement each other (max. 5 lines per partner).

DTU Vet, Research group for Bacteriology and Pathology (partner 1) primarily works with bacterial diseases, e.g. in aquaculture, where the research is focused on disease description, development and dissemination, possible preventive measures of known as well as new diseases, studies of antibiotic resistance and development of new diagnostic methods. The group has a huge experience with experimental infection models with bacteria, especially F. psychrophilum but also other fish pathogens.

DTU Vet, Research group for Microbial Ecology and Animal Health (partner 2) has a large experience within the field of interaction between feed, management and intestinal microbiota in different animals. This partner has implemented many molecular methods for characterization of the intestinal microbiota and the necessary advanced state-of-the-art equipment (laser microdissection microscope (PALM), confocal laser scannings microscopy (Zeiss LSM 710) and next generation sequencing (Ilumina/Solexa Genome Analyzer II and Roche Genome Sequencer FLX Titanium System) is available at the institute.

KU Life, Laboratory of Aquatic Pathobiology (partner 3) conducts basic, applied and strategic research within the field of pathogen-host interactions and immunology in fish. The laboratory uses a long range of techniques with special relevance for this project. They employ immunohistochemistry, quantitative RT-PCR (QPCR), ELISA, and Western blot. The research field is primarily the immune system of the rainbow trout.

BioMar A/S (partner 4), fish feed company with sections in Brande and Hirtshals. The company develops feed for aquaculture worldwide and has in later years worked with the interaction between feed and the immune defence of the fish. BioMar participates in the project by making their knowledge within the field available and will produce the different experimental diet types used in the project (see WP 2).

Danish Aquaculture organization (partner 5) participates in the project by making their knowledge within practical aquaculture available.

A12.11. Expected collaboration with other research institutions/companies nationally and internationally (max. ½ page).

Internationally, the OPTIFISH participants have contact with a group from the Norwegian School of Veterinary Science, Institute of Basic Sciences and Aquatic Medicine, The Aquaculture Protein Centre, in Oslo. These researchers from Norway have the best international background for studying relations between diet and intestinal pathology in salmon.

A12.12. The relation to previous projects within the projects focus areas (if any) including references to these (max. ½ page).

Lone Madsen and Inger Dalsgaard have been participating scientists in the project Oraqua (FØJO III project) ending ultimo 2010. The focus has been 1) monitoring of fish health in organic fish farming, 2) testing the effect of different diets on an experimental challenge with the fish pathogen Yersinia ruckeri.

References:

Jokumsen A, Lund I, Dalsgaard AJT, Dalsgaard I, Nielsen HH, Rasmussen HT, Larsen VJ, Jessen PB & Holm J (2009). Proteinafgrøder til økologiske regnbueørreder (Oncorhynchus mykiss). Poster presented at: Økologi-kongres. Odense, Danmark

Madsen L & Dalsgaard I (2010). Yersinia ruckeri challenge on rainbow trout fed different diet types. Sixth International Symposium on Aquatic Animal Health, Tampa, USA, 5.-9. september. Poster presentation

A13. Tables with milestones and deliverables with information as requested in the table in A16.

WP1 M 1 Homepage for the project established
WP1 M 2 Project meetings with all project partners
WP1 M 3 Status reports 2011, 2012, 2013, all

WP1 M 4 Project completion, final report, dissemination of results
WP1 M 5 Publication of results in scientific journals
WP1 M 6 Dissemination of results to the aquaculture industry
WP2 M 7 Feed experiments with different organic diet types on experimental fish completed
WP2 M 8 Fish samples provided to other work packages
WP3 M 9 Analyses of the microbiota by bacteriology and next generation sequencing
WP3 M 10 Metagenomic analysis of the microbiota
WP3 M 11 Fluorescent in situ hybridization for detection of pathogens and probiotic bacteria
WP3 M 12 Laser capture microdissection for identification of bacteria in intestinal lesions
WP4 M 13 Antibody response and immune gene expression profiles in the different diet groups characterized
WP5 M 14 Experimental infection trials of rainbow trout fry with bacterial pathogens
WP5 M 15 Clinical samples from experimental trials analyzed, and pathogens identified.

A14. List of deliverables from the project (also fill out the table in A17)

D1 Scientific article, composition of the microbiota in relation to diet type and disease
D2 Scientific article, host response in relation to diet type and disease
D3 Scientific article, fish health/disease in relation to diet type
D4 Scientific article concerning the most important results of the project
D5 Presentation of results at meetings and congresses
D6 M.Sc. students
D7 Commercialization of a new standard diet for organic rainbow trout fry
D8 Articles in popular journals
D9 Dissemination of results and information through website
D10 Oral presentations targeting end users
D11 Management of the project, project meetings and annual reports

A15. List of appendices

Budget form (B)
Participation forms (D)
CVs
Main references

A16. Milestones and time schedule for the entire project

wp no.	Milestone no.	Title/activity	Responsible project participant	Date/year	Other participants
WP1	M 1	Homepage for the project established	Partner 1	Jan/2011	Partner 2, 3
WP1	M 2	Project meetings with all project partners	Partner 1	Dec / 2013	Partner 2, 3, 4, 5
WP1	M 3	Status reports 2011, 2012, 20113	Partner 1	2011, 2012, 2013	Partner 2, 3, 4, 5
WP 1	M 4	Project completion, final repost, dissemination of results	Partner 1	Dec/2013	Partner 2, 3, 4, 5
WP1	M 5	Publication of results in scientific journals	Partner 1, 2, 3	Dec/2013	
WP1	M 6	Dissemination of results to the aquaculture industry	Partner 1	Oct/2013	Partner 2, 3,5
WP2	M 7	Feed experiments with different organic diet types on experimental fish completed	Partner 4	Dec 2012	Partner 1
WP2	M 8	Fish samples provided to other work packages	Partner 1	Dec 2012	Partner 2, 3, 4
WP3	M 9	Analyses of the microbiota by bacteriology and next generation sequencing	Partner 2	Feb/2013	Partner 1
WP3	M10	Metagenomic analysis of the microbiota	Partner 2	Sept/2013	Partner 1
WP3	M 11	Fluorescent in situ hybridisation for detection of pathogens and probiotic bacteria	Partner 2	Sept/2013	Partner 1
WP3	M 12	Laser capture microdissection for identification of bacteria in intestinal lesions	Partner 2	Sept/2013	Partner 1
WP4	M 13	Antibody response and immune gene expression profiles in different diet groups characterised	Partner 3	Sept/2013	Partner 1
WP5	M 14	Experimental infection trials of rainbow trout fry with bacterial pathogens	Partner 1	June/2013	Partner 4
WP5	M 15	Clinical samples from experimental trials analyzed and pathogens identified	Partner 1	Sept/2013	Partner 2, 3

A17. List over deliverables (D=deliverables) for the entire project, stating whether the deliverable belongs to the research part of the project (R); the development part (D); and/or demonstration (Dm).

D. no.	Deliverable	Responsible project participant	Date/year	R, D, or Dm Effective working time, months [1]	Type of deliverable*
D1	Scientific article composition of the microbiota in relation to diet type and disease	MB	2013	R/18	S1
D2	Scientific article host response in relation to diet type and disease	KB	2013	R/15	S1
D3	Scientific article fish health/disease in relation to diet type	LM/ID	2012	R/17	S1
D4	Scientific article concerning the most important results of the project	LM	2013	R/6	S2
D5	Presentation of results at meetings and congresses	all	2011/12/13	R/2	S4
D6	MSc students	all	2011-12	R/4	C1
D7	Commercialization of a new standard diet for organic rainbow trout fry	AHL	2013	R/2	C5
D8	Articles in popular journals	LM/ID	2012/2013	R/2	P1
D9	Dissemination of results and information through website	LM	2012	R/1	P1
D10	Oral presentations targeting end users	LM/ID/AHL/NHH	2011/12/13	R/2	P2
D11	Management of the project, project meetings and annual reports	LM	2011/12/13	R/3	?

* Fill in the type of deliverable. Use the List of type of deliverables on the last page in Annex 3 “Instructions for filling in the application form”.

[1] The total amount of months must be consistent with the total number of months in the budgets, and will therefore show the relative working effort per work package.